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(54) Title: CANDIDA HEAT SHOCK PROTEIN, cDNA AND USES THEREOF

(57) Abstract

A nucleotide sequence and related protein from Candida homologous to 70 kd heat shock protein, for uses in diagnosis and therapy.

INTERNATIONAL SEARCH REPORT

International application No.
[redacted]/IT 96/00097

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Futher Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

CANDIDA HEAT SHOCK PROTEIN, cDNA AND USES THEREOF

The invention concerns the cDNA and the corresponding protein of a heat shock protein isolated from C. albicans, and fragments thereof to develop methods to identify C. albicans in biological and/or environment samples, and/or preparations either for therapeutic, prophylaxis or vaccine purpose.

Pathogenic yeasts are the major agents of opportunistic infections in immunosuppressed patients, in particular AIDS, tumor, neutropenia patients or bone marrow transplanted subjects (1). HIV$^+$ subject susceptibility to C. albicans is related to the strong decrease of cell-mediated immunity because of the numerical and functional decrease of CD4$^+$ helper-inducer lymphocytes (2).

C. albicans cell wall mannoproteins and heatshock proteins of other microorganisms as well, are major antigens and immunomodulators, and play a relevant role during host invasion and infection (3,4).

By using a rabbit immune serum obtained against heat-inactivated C. albicans ATCC 20955 strain cells, the authors of the instant invention isolated the caRLV130 clone from an expression library in the λgt11 phage obtained by cDNA isolated from C. albicans at the yeast growth stage. Said clone contains a DNA insert of 2325 base pairs which codes in the 5'-3' direction from +105 to +2072 for a 656 aminoacid protein having a strong homology with a S. cerevisiae heat shock protein 70.

HSPs are induced by different stresses, either chemical or physical, normally by heating. Many HSPs are present and active also in non stressed cells, where they play important functions of cell physiology ("chaperonins"). They may be grouped in families of

different molecular weights, very conserved even among phylogenesis distant organisms (5). Therefore it should not be surprising either that HSPs are involved in the immune response, or that they represent major antigens of different pathogenic agents, or that they may give autoimmune responses, given to the fact that the infection itself represents an extreme form of stress, both for the infectious agent and for the host (4).

It is therefore an object of the invention a nucleic acid comprising a nucleotide sequence coding the protein having the amino acid sequence of SEQ ID No.2 or parts thereof. Preferably the nucleic acid comprises a nucleotide sequence with at least a 65% homology with the nucleotide sequence of SEQ ID No.1 or parts thereof. More preferably the nucleic acid comprises the nucleotide sequence of SEQ ID No.1 or parts thereof.

Further object of the invention is a composition comprising a nucleic acid comprising a nucleotide sequence coding the protein having the amino acid sequence of SEQ ID No.1 or parts thereof. Preferably the composition comprises a nucleic acid having a nucleotide sequence with at least a 65% homology with the nucleotide sequence of SEQ ID No.1 or parts thereof. More preferably the composition comprises a nucleic acid having the nucleotide sequence of SEQ ID No.1 or parts thereof.

Further object of the invention is the use of the nucleic acid comprising a nucleotide sequence coding the protein having the amino acid sequence of SEQ ID No.2 or parts thereof for oligonucleotide probes to be used in diagnosis and typing of Candida related pathologies. The use of a nucleic acid having at least a 65% homology with the nucleotide sequence of SEQ ID No.1 or parts thereof is preferred. The use of a nucleic acid having the

nucleotide sequence of SEQ ID No.1 or parts thereof is most preferred.

The oligonucleotides of the invention are advantageously used for PCR (polymerase chain reaction) to detect the presence in biological and/or environment samples either of C. albicans or of other Candida species or of yeast-like related microorganisms comprising said gene; in a labeled form (radionuclides, biotin, enzymes, etc.) to detect the presence in biological and/or environment samples either of C. albicans or of other related; for the C. albicans or related species typing and/or diagnosis; as potential antibiotic and/or chemiotherapic targets, or antisense RNA active for Candida species and/or yeast-like related microorganisms coding an homologous sequence.

Another object of the invention is a polypeptide having the aminoacid sequence comprised in the SEQ ID No.2, or having at least a 50% homology with SEQ ID No. 2 or fragments, and/or functional and immunologic homologous thereof.

Further object of the invention is a composition comprising a polypeptide having an amino acid sequence comprised in SEQ ID No.2 or having at least a 50% homology with SEQ ID No. 2 or fragments, and/or functional and immunologic homologous thereof.

Further object of the invention is the use of a polypeptide having the amino acid sequence comprised in SEQ ID No.2 or having at least a 50% homology with SEQ ID No. 2 or of fragments, and/or functional and/or immunologic homologous thereof to make polyclonal or monoclonal antibodies against the 70 kd heat shock protein (HSP70) of C. albicans or related species.

Further object of the invention is the use of a polypeptide having the amino acid sequence comprised in

SEQ ID No.2 or having at least a 50% homology with SEQ ID No. 2 or of fragments, and/or functional and/or immunologic homologous thereof to detect C. albicans and related species HSP70 in a biological sample having a human, animal or environmental origin.

Further object of the invention is the use of a polypeptide having the amino acid sequence comprised in SEQ ID No.2 or having at least a 50% homology with SEQ ID No. 2 or of fragments, and/or functional and/or immunologic homologous thereof for the preparation of a composition to be used for prophylaxis and/or therapy of C. albicans or related microorganisms (pathogenic yeasts) diseases.

Further object of the invention is the use of a polypeptide having the amino acid sequence comprised in SEQ ID No.2 or having at least a 50% homology with SEQ ID No. 2 or of fragments, and/or as potential antibiotic and/or chemiotherapic targets active for Candida species and/or yeast-like related microorganisms coding an homologous sequence.

The invention will be described in different embodiments for clarifying but not limiting purposes.

Figure 1 represents the 1971 base pair DNA sequence (small letters) corresponding to the open reading frame of λgt11-(caRLA130) clone insert and deduced aminoacid sequence (capital letters one-letter code).

Figure 2 represents the nucleotide sequence of the coding insert of caRLV130 clone (small letter) and comparison with S. cerevisiae YSCSSA1 gene (capital letter).

Figure 3 represents the 656 aminoacid sequence deduced from the coding insert of caRLV130 clone (small letter) and comparison with the S. cerevisiae YSCSSA1

gene (capital letter). The aminoacid code utilized is the one letter code.

Figure 4 represents in panel A. Southern blot analysis of C. albicans strain ATCC 20955 chromosomes, obtained by pulse field electrophoresis (TAFE). The caRLV130 probe labeling refers to the highest molecular weight chromosome (3.5 Mbp). In panel B. Electrophoretic separation of C. albicans strain ATCC 20955 chromosomes.

Figure 5 represents on the left side: Northern blot analysis by hybridization of total RNA extracted from C. albicans cells grown at 22°C and transferred at 37°C for the time indicated with radiolabeled caRLV130 (cahsp70) and actin probes. The actin probe hybridization was performed to control the RNA amount on filters (see ref. 8). On the right side: immunoblotting reactivity of anti-CAHSP70 mouse serum with C. albicans extracts, at different times further to inducing a heat shock response as previously described.

Figure 6 represents in panel A. SDS-PAGE analysis: a) expression products of E. coli M15 containing the pDS56/RBS-E$^-$-6his caRLV130/1 plasmid; b) expression products of E. coli M15 containing the pDS56/RBS-E$^-$-6his caRLV130/2 plasmid; c) expression products of E. coli M15 containing the pDS56/RBS-E$^-$-6his caRLV130/3 plasmid; d) expression products of E. coli M15 containing the pDS56/RBS-E$^-$-6his caRLV130/4 plasmid. N.I.: Non induced E. coli culture extracts. I.: 1 mM IPTG induced E. coli culture extracts. P.: Purified fraction on histidine affinity nickel column from 1 mM IPTG induced E. coli culture extracts. In panel B. Schematic representation of caRLV130 coding sequence portions cloned into recombinant plasmids used in panel A. Right side: molecular weight in kDa. Left side: denomination of the expression product of recombinant plasmid. For further details, see table I.

Figure 7 represents the reactivity after immunoblotting on nitrocellulose filters of mouse sera as shown in the figure obtained against CAHSP70 fragments; a) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/1 plasmid in 1 mM IPTG induced E. coli; b) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/2 plasmid in 1 mM IPTG induced M15 E. coli; c) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/3 plasmid in 1 mM IPTG induced M15 E. coli; d) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/4 plasmid in 1 mM IPTG induced M15 E. coli (see also Fig. 6 and table I for a definition of polypeptide fragments). Left side: molecular weight of purified fragments.

Figure 8 represents the reactivity after immunoblotting on nitrocellulose filters of wealthy human sera obtained against CAHSP70 and fragments thereof; a) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/1 plasmid in 1 mM IPTG induced M15 E. coli; b) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/2 plasmid in 1 mM IPTG induced M15 E. coli; c) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/3 plasmid in 1 mM IPTG induced M15 E. coli; d) expression products of nickel column purified pDS56/RBSII-E⁻-6his caRLV130/4 plasmid in 1 mM IPTG induced M15 E. coli. Left side: molecular weight of purified fragments. Right side: denomination of purified protein fragments. For further details see also table I.

Figure 9 represents in panel A. PCR experiment performed using oligonucleotide combination CA2-CA3 in the presence of C. albicans, C. parapsilosis (2), C. glabrata (3), C. guillermondii (4), C. krusei (5), C.

tropicalis (6), Mus muris (7), E. coli (8), S. cerevisiae (9) DNAs. Control with no DNA is as (10). At the right side the molecular weight of the amplified fragment is indicated. In panel B. PCR experiment using the combination of CA1-CA4 oligonucleotides in the presence of C. albicans cDNA: DNA amplified from C. albicans DNA: 10 ng (2); 1 ng (3); 100 pg (4); 10 pg (5); 1 pg (6). Control: reaction with no DNA (1). PCR reaction conditions are as follows: 90 sec. 94°C denaturation; 90 sec. 60°C annealing; 120 sec. 72°C extension; 25 cycles.

Figure 1 shows the 1971 bp coding region of the isolated gene.

The caRLV130 sequence was filed with EMBL data base (No. Z30210). No intron can be found in the intronic sequence, as shown by PCR product analysis and by "Southern-blot". By comparing the caRLV130 insert sequence with sequences present in the 6.7 version "GENE BANK" data base, some homologies can be detected. The insert shows the most high homology with the S. cerevisiae gene SSA1 (one of the nine heatshock yeast gene family). The overall nucleotide sequence homology is of 78.8% in the coding region (figg. 2 and 3).

The gene corresponding to the caRLV130 sequence was mapped on the C. albicans chromosome showing the highest molecular weight (3.5 Mpb) by pulse field electrophoresis (transverse-alternate: TAFE) utilizing the caRLV130 labeled cDNA insert as hybridization probe with C. albicans chromosomes blotted on nitrocellulose filters (fig. 4A an 4B). Gene transcription is activated by exposing cells to a temperature higher than room temperature (thermal shift from 22°C to 37°C). Such finding was demonstrated by hybridization experiments using C. albicans total RNA (from cells grown either at 22°C or at 37°C, fractionated according to molecular

weight on formaldehyde agarose gel and blotted on nitrocellulose filters) and the caRLV130 DNA insert as radioactive probe. The induction of transcription is coupled also to an increase of protein expression, 2, 6 and 24 hours further to the 22°C to 37°C temperature shift (see fig. 5).

Different portions of the caRLV130 insert sequence were cloned in the expression plasmid pDS56/RBSII-E⁻-6his (6), and coded polypeptides were expressed in E. coli after fusion of their amino terminal sequence with 6 histidine residues. The histidine stretch allowed to a rapid and efficient purification of polypeptides derived from the caRLV130 insert sequence on nickel columns (see fig. 6 and table I for denomination and length of polypeptide fragments).

Table I

Definition of nickel column purified CAHSP70 polypeptides. Peptides are coded by pDS56/RBSII-E⁻-6his recombinant plasmids wherein caRLV130 fragments were cloned. The position refers to nucleotide and aminoacid sequences as shown in Fig. 1. The peptide length refers to the fusion product coded by the recombinant plasmid.

coding DNA				coded peptide				
denomination	sequence location on cDNA	position (nt)	length (bp)	denomination	peptide fragment location	position (aa)	length	
caRLV130/1	whole coding	1-2229	2229	CAHSP70	whole protein	1-656	71.3	664
caRLV130/2	3′ end cDNA	1393-2229	837	CAHSP70/2	C-terminus	465-656	21.0	202
caRLV130/3	5′ end cDNA	1-732	732	CAHSP70/3	N-terminus	1-244	28.4	261
caRLV130/4	5′ end cDNA	1-1027	1027	CAHSP70/4	N-terminus	1-342	39.4	358

After purification, recombinant peptides were used as immunogens to produce mouse immune sera and are therefore able also to induce monoclonal antibodies. Therefore, according the immunization schedule shown in table II, polypeptides, and the whole purified protein as well, induce specific antibodies in a 18-22 g weight Balb/c mouse.

Table II

Immunization schedule of 18-22 g weight Balb/c mice with CAHSP70 peptides purified as described in the text and in Fig.6.

Immunogen	Immunization (day 1)	First boost (day 21)	Second boost (day 41)	Serum titer (day 51)
CAHSP70	5 µg	5 µg	10 µg	> 12.800
CAHSP70/2	5 µg	5 µg	10 µg	> 12.800
CAHSP70/3	5 µg	5 µg	10 µg	> 12.800
CAHSP70/4	5 µg	5 µg	10 µg	> 12.800

The indicated immunogen concentration was inoculated intraperitoneally in a 200 µl volume.
The titer was determined by indirect ELISA with the antigen used for coating at a 200 ng/well concentration, in a final volume of 100 µl, and represents the highest serum dilution able to give an ELISA positive reaction (optical density at 405 nm ≥ two fold the no antigen control value).

Serum titers for each antigen resulted to be > 12.800 by immunoenzyme test (indirect ELISA) with the adsorbed antigen at 200 ng/well, in a final volume of 100 µl. The specificity of immunoenzyme test results were confirmed in immunoblot experiments on nitrocellulose filters, as shown in Fig. 7.

The same polypeptides were utilized as immunogens in proliferation assays on peripheral human blood lymphocytes by evaluating the ^{3}H-thymidine uptake further to 7 day culturing according to standard techniques (7).

Results obtained with different donors (two examples are shown in table III) demonstrate that CAHSP70 is able to induce a good thymidine uptake and the proliferation of naive lymphocytes from umbilical cord blood (Table IV), suggesting that the protein itself or parts thereof has a mitogenic activity.

Table III

Peripheral blood lymphocytes proliferation induction activity of CAHSP70 and fragments thereof

inducing materials	dose	lymphoproliferative activity ^{3}H-thymidine uptake (cpm ± SD/2x10^4 cells)
none	-	500 ± 200
MP-F2	50 µg/ml	13.393 ± 11.555
IL-2	100 U/ml	28.205 ± 18.014
CAHSP70	1 µg/ml	8.730 ± 5.181
CAHSP70/2	1 µg/ml	2.900 ± 2.300
CAHSP70/3	1 µg/ml	3.600 ± 2.700
CAHSP70/4	1 µg/ml	11.685 ± 8.174

Lymphoproliferation of wealthy donor peripheral blood lymphomonocyte cultures further to induction with the CAHSP70 cloned fragments. Positive controls: C. albicans mannoproteic antigen (MP-F2) and Interleukin-2 (IL-2). Negative controls: no materials. Shown values represent average values ± SD from 7 experiments with 5 different donors. ^{3}H-thymidine uptake was determined after 7 days of culture. For technical details, see ref. 7.

Table IV

Umbilical cord blood cell proliferation induction activity of CAHSP70 and fragments thereof

inducing materials	dose	lymphoproliferative activity ^{3}H-thymidine uptake (cpm ± SD/2x10^4 cells)	
		cord blood 1	cord blood 2
none		2.5 ± 0.4	1.3 ± 0.3
IL-2	100 U/ml	37.7 ± 4.5	32.8 ± 6.0
MP-F2	50 µg/ml	3.0 ± 1.4	1.5 ± 0.4
CAHSP70	1 µg/ml	12.5 ± 1.8	22.8 ± 6.6
CAHSP70/2	1 µg/ml	18.2 ± 3.0	23.1 ± 3.9
CAHSP70/3	1 µg/ml	23.8 ± 5.4	20.6 ± 9.2
CAHSP70/4	1 µg/ml	14.8 ± 3.9	17.2 ± 1.7

Proliferation of two donor umbilical cord blood cultures further to induction with the CAHSP70 cloned fragments. Positive controls: C. albicans mannoproteic antigen (MP-F2) and Interleukin-2 (IL-2). Negative controls: no materials. Shown values represent average values ± SD from 3 wells. For technical details, see table III legend and ref. 7.

Furthermore, immunoblotting experiments revealed the presence of anti-CAHSP70 antibodies in sera from adult wealthy humans, and in particular of the anti-CAHSP70/4 fragment (Fig. 8), suggesting that this fragment contains the immunodominant epitope. Taken together, lymphoproliferations human serum immunoblotting data suggest inequivocabilly that CAHSP70 is recognized by the immune system during the Candida usual colonization of healthy subjects.

Moreover, in immunoblotting on nitrocellulose filters, anti-CAHSP70 murine sera recognize more than one component of the HSP70 family from heat induced C. albicans extracted proteins (Fig. 5), thus showing that the expression product of caRLV130 insert is a C.

albicans protein which is expressed after the heat shock. According to the above results we named as CAHSP70 the C. albicans protein having the following properties: I) it comprises the aminoacid sequence coded by the caRLV130 insert; II) its gene maps on C. albicans chromosome 1 (having the highest molecular weight); III) its expression is induced by temperature shift; IV) it induces specific antibodies able to recognize cloned and purified fragments (subunits); V) it induces a lymphoproliferation in lymphomonocytic cultures from peripheral human blood. The relevant gene was named as cahsp70.

The CAHSP70 cloning, and its molecular and biochemical characterization, allows to develop a diagnostic molecular method based upon the amplification of DNA inserts corresponding to caRLV130, other than immunological studies of C. albicans 70 kDa heat shock protein expression. According to the caRLV130 insert sequence, we have synthesized oligonucleotides which were utilized for polymerase chain reaction (PCR) experiments, to analyze their ability to amplify DNA fragments which are homologous to C. albicans caRLV130 DNA. Two oligonucleotides (CA2-CA4) were chosen in the regions showing the minimal homology between the caRLV130 cDNA sequence and known HSP70 coding gene sequences (see Fig. 2 for the caRLV130 and YSCSSA1 sequence aligning, see Table V for the definition of minimal homology regions and Table VI for the sequence of oligonucleotides which were utilized for the assay).

The combination of CA2 (GAAATGAAAGATAAGATTGGTGCA) and CA3 (CCACAGTAAATTACCTATTTCTTCCTC) oligonucleotides is able to amplify DNA fragments having the expected size and a sequence specific of C. albicans DNA (Fig. 9A), whereas the assay sensitivity is shown in Fig. 9B by

using CA1 (ATGTCTAAAGCTGTTGGTATTG) and CA4 (CTGCACCAATCTTATCTTTCATTTCACCATCATT) oligonucleotides.

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SEQUENCE LISTING

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(E) COUNTRY: Italy
(F) POSTAL CODE (ZIP): 00184

(ii) TITLE OF INVENTION: Candida heath shock protein, gene and uses thereof

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2001 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1968

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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 1               5                  10                  15

GCT CAT TTT GCC AAT GAT AGA GTT GAA ATT ATT GCT AAT GAT CAA GGT        96
Ala His Phe Ala Asn Asp Arg Val Glu Ile Ile Ala Asn Asp Gln Gly
            20                  25                  30

AAT AGA ACT ACC CCT TCA TTT GTT GCC TTC ACT GAT ACT GAA AGA TTG       144
Asn Arg Thr Thr Pro Ser Phe Val Ala Phe Thr Asp Thr Glu Arg Leu
        35                  40                  45

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65                  70                  75                  80

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Val Ile Asn Asp Ala Lys His Phe Pro Phe Lys Val Ile Asp Lys Ala
                85                  90                  95

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            100                 105                 110

TCA CCA GAA GAA ATT TCT TCA ATG GTT TTA ACA AAA ATG AAA GAA ATT       384
Ser Pro Glu Glu Ile Ser Ser Met Val Leu Thr Lys Met Lys Glu Ile
        115                 120                 125

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GCT GAT GCC AAG ATT GAT AAA TCT CAA GTT GAA GAA ATT GTC TTG GTT     1008
Ala Asp Ala Lys Ile Asp Lys Ser Gln Val Glu Glu Ile Val Leu Val
                325                 330                 335

GGT GGG TCC ACC AGA ATT CCA AAG ATT CAA AAA TTG GTT TCT GAT TTC     1056
Gly Gly Ser Thr Arg Ile Pro Lys Ile Gln Lys Leu Val Ser Asp Phe
            340                 345                 350
```

```
TTT AAT GGT AAA GAA TTG AAT AAA TCT ATC AAC CCT GAT GAA GCT GTT      1104
Phe Asn Gly Lys Glu Leu Asn Lys Ser Ile Asn Pro Asp Glu Ala Val
        355                 360                 365

GCT TAT GGT GCT GCT GTT CAA GCT GCC ATT TTA ACT GGT GAT ACT TCT      1152
Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Thr Gly Asp Thr Ser
    370                 375                 380

TCC AAG ACT CAA GAT ATT TTG TTA TTG GAT GTT GCT CCA TTG TCA TTA      1200
Ser Lys Thr Gln Asp Ile Leu Leu Leu Asp Val Ala Pro Leu Ser Leu
385                 390                 395                 400

GGT ATT GAA ACT GCT GGT GGT ATC ATG ACC AAA TTG ATT CCA AGA AAT      1248
Gly Ile Glu Thr Ala Gly Gly Ile Met Thr Lys Leu Ile Pro Arg Asn
                405                 410                 415

TCT ACT ATT CCA ACT AAG AAA TCA GAA ACT TTC TCC ACT TAT GCC GAT      1296
Ser Thr Ile Pro Thr Lys Lys Ser Glu Thr Phe Ser Thr Tyr Ala Asp
            420                 425                 430

AAC CAA CCA GGT GTT TTG ATT CAA GTG TTT GAA GGT GAA AGA GCT AAA      1344
Asn Gln Pro Gly Val Leu Ile Gln Val Phe Glu Gly Glu Arg Ala Lys
        435                 440                 445

ACT AAA GAT AAC AAC TTG TTG GGT AAA TTT GAA TTA TCT GGT ATT CCA      1392
Thr Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Ser Gly Ile Pro
    450                 455                 460

CCA GCT CCA AGA GGC GTC CCT CAA ATT GAA GTT ACT TTC GAT ATT GAT      1440
Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile Asp
465                 470                 475                 480

GCT AAT GGT ATC TTG AAT GTT TCT GCT TTA GAA AAA GGT ACT GGT AAA      1488
Ala Asn Gly Ile Leu Asn Val Ser Ala Leu Glu Lys Gly Thr Gly Lys
                485                 490                 495

ACT CAA AAG ATT ACT ATC ACC AAC GAT AAA GGT AGA TTA TCC AAA GAA      1536
Thr Gln Lys Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser Lys Glu
            500                 505                 510

GAA ATT GAT AAA ATG GTT AGT GAA GCT GAA AAA TTC AAA GAA GAA GAT      1584
Glu Ile Asp Lys Met Val Ser Glu Ala Glu Lys Phe Lys Glu Glu Asp
        515                 520                 525
```

```
GAA AAG GAA GCT GCT AGA GTC CAA GCC AAG AAT CAA TTG GAA TCT TAT      1632
Glu Lys Glu Ala Ala Arg Val Gln Ala Lys Asn Gln Leu Glu Ser Tyr
    530                 535                 540

GCT TAT TCA TTG AAA AAC ACA ATC AAT GAT GGT GAA ATG AAA GAT AAG      1680
Ala Tyr Ser Leu Lys Asn Thr Ile Asn Asp Gly Glu Met Lys Asp Lys
545                 550                 555                 560

ATT GGT GCA GAT GAT AAA GAA AAA TTA ACT AAA GCC ATT GAT GAA ACT      1728
Ile Gly Ala Asp Asp Lys Glu Lys Leu Thr Lys Ala Ile Asp Glu Thr
                565                 570                 575

ATT TCT TGG TTA GAT GCA TCT CAA GCT GCT TCT ACT GAA GAA TAC GAA      1776
Ile Ser Trp Leu Asp Ala Ser Gln Ala Ala Ser Thr Glu Glu Tyr Glu
            580                 585                 590

GAT AAA CGT AAA GAA TTA GAA TCA GTT GCT AAT CCA ATC ATT AGT GGT      1824
Asp Lys Arg Lys Glu Leu Glu Ser Val Ala Asn Pro Ile Ile Ser Gly
        595                 600                 605

GCT TAT GGT GCT GCC GGT GGC GCT CCA GGT GGT GCA GGC GGA TTC CCA      1872
Ala Tyr Gly Ala Ala Gly Gly Ala Pro Gly Gly Ala Gly Gly Phe Pro
    610                 615                 620

GGT GCT GGT GGC TTC CCA GGT GGT GCC CCA GGT GCC GGT GGT CCA GGT      1920
Gly Ala Gly Gly Phe Pro Gly Gly Ala Pro Gly Ala Gly Gly Pro Gly
625                 630                 635                 640

GGT GCT ACT GGT GGT GAA TCT AGT GGA CCA ACT GTT GAA GAA GTT GAT      1968
Gly Ala Thr Gly Gly Glu Ser Ser Gly Pro Thr Val Glu Glu Val Asp
                645                 650                 655

TAAATGAGGAAGAAATAGGTAATTTACTGTGG                                    2000
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 656 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Ser Lys Ala Val Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val
 1               5                  10                  15
Ala His Phe Ala Asn Asp Arg Val Glu Ile Ile Ala Asn Asp Gln Gly
            20                  25                  30
Asn Arg Thr Thr Pro Ser Phe Val Ala Phe Thr Asp Thr Glu Arg Leu
        35                  40                  45
Ile Gly Asp Ala Ala Lys Asn Gln Ala Ala Met Asn Pro Ala Asn Thr
    50                  55                  60
Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Lys Phe Asp Asp Pro Glu
65                  70                  75                  80
Val Ile Asn Asp Ala Lys His Phe Pro Phe Lys Val Ile Asp Lys Ala
                85                  90                  95
Gly Lys Pro Val Ile Gln Val Glu Tyr Lys Gly Glu Thr Lys Thr Phe
            100                 105                 110
Ser Pro Glu Glu Ile Ser Ser Met Val Leu Thr Lys Met Lys Glu Ile
        115                 120                 125
Ala Glu Gly Tyr Leu Gly Ser Thr Val Lys Asp Ala Val Val Thr Val
    130                 135                 140
Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Asp Ala Gly
145                 150                 155                 160
Thr Ile Ala Gly Leu Asn Val Leu Arg Ile Ile Asn Glu Pro Thr Ala
                165                 170                 175
Ala Ala Ile Ala Tyr Gly Leu Asp Lys Lys Gly Ser Arg Gly Glu His
            180                 185                 190
Asn Val Leu Ile Phe Asp Leu Gly Gly Gly Thr Phe Asp Val Ser Leu
        195                 200                 205
Leu Ala Ile Asp Glu Gly Ile Phe Glu Val Lys Ala Thr Ala Gly Asp
    210                 215                 220
Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg Leu Val Asn Phe Phe
225                 230                 235                 240
Ile Gln Glu Phe Lys Arg Lys Asn Lys Lys Asp Ile Ser Thr Asn Gln
                245                 250                 255
Arg Ala Leu Arg Arg Leu Arg Thr Ala Cys Glu Arg Ala Lys Arg Thr
            260                 265                 270
Leu Ser Ser Ser Ala Gln Thr Ser Ile Glu Ile Asp Ser Leu Tyr Glu
        275                 280                 285
Gly Ile Asp Phe Tyr Thr Ser Ile Thr Arg Ala Arg Phe Glu Glu Leu
```

290 295 300

Cys Ala Asp Leu Phe Arg Ser Thr Leu Asp Pro Val Gly Lys Val Leu
305 310 315 320

Ala Asp Ala Lys Ile Asp Lys Ser Gln Val Glu Glu Ile Val Leu Val
325 330 335

Gly Gly Ser Thr Arg Ile Pro Lys Ile Gln Lys Leu Val Ser Asp Phe
340 345 350

Phe Asn Gly Lys Glu Leu Asn Lys Ser Ile Asn Pro Asp Glu Ala Val
355 360 365

Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Thr Gly Asp Thr Ser
370 375 380

Ser Lys Thr Gln Asp Ile Leu Leu Leu Asp Val Ala Pro Leu Ser Leu
385 390 395 400

Gly Ile Glu Thr Ala Gly Gly Ile Met Thr Lys Leu Ile Pro Arg Asn
405 410 415

Ser Thr Ile Pro Thr Lys Lys Ser Glu Thr Phe Ser Thr Tyr Ala Asp
420 425 430

Asn Gln Pro Gly Val Leu Ile Gln Val Phe Glu Gly Glu Arg Ala Lys
435 440 445

Thr Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Ser Gly Ile Pro
450 455 460

Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile Asp
465 470 475 480

Ala Asn Gly Ile Leu Asn Val Ser Ala Leu Glu Lys Gly Thr Gly Lys
485 490 495

Thr Gln Lys Ile Thr Ile Thr Asn Asp Lys Gly Arg Leu Ser Lys Glu
500 505 510

Glu Ile Asp Lys Met Val Ser Glu Ala Glu Lys Phe Lys Glu Glu Asp
515 520 525

Glu Lys Glu Ala Ala Arg Val Gln Ala Lys Asn Gln Leu Glu Ser Tyr
530 535 540

Ala Tyr Ser Leu Lys Asn Thr Ile Asn Asp Gly Glu Met Lys Asp Lys
545 550 555 560

Ile Gly Ala Asp Asp Lys Glu Lys Leu Thr Lys Ala Ile Asp Glu Thr
565 570 575

Ile Ser Trp Leu Asp Ala Ser Gln Ala Ala Ser Thr Glu Glu Tyr Glu
580 585 590

Asp Lys Arg Lys Glu Leu Glu Ser Val Ala Asn Pro Ile Ile Ser Gly
595 600 605

Ala Tyr Gly Ala Ala Gly Gly Ala Pro Gly Gly Ala Gly Gly Phe Pro

```
      610                 615                 620
Gly Ala Gly Gly Phe Pro Gly Gly Ala Pro Gly Ala Gly Gly Pro Gly
625                 630                 635                 640
Gly Ala Thr Gly Gly Glu Ser Ser Gly Pro Thr Val Glu Glu Val Asp
                    645                 650                 655
```

Claims

1. A nucleic acid comprising a nucleotide sequence coding the protein having the amino acid sequence of SEQ ID No.2 or parts thereof.

2. A nucleic acid comprising a nucleotide sequence with at least a 65% homology with the nucleotide sequence of SEQ ID No.1 or parts thereof.

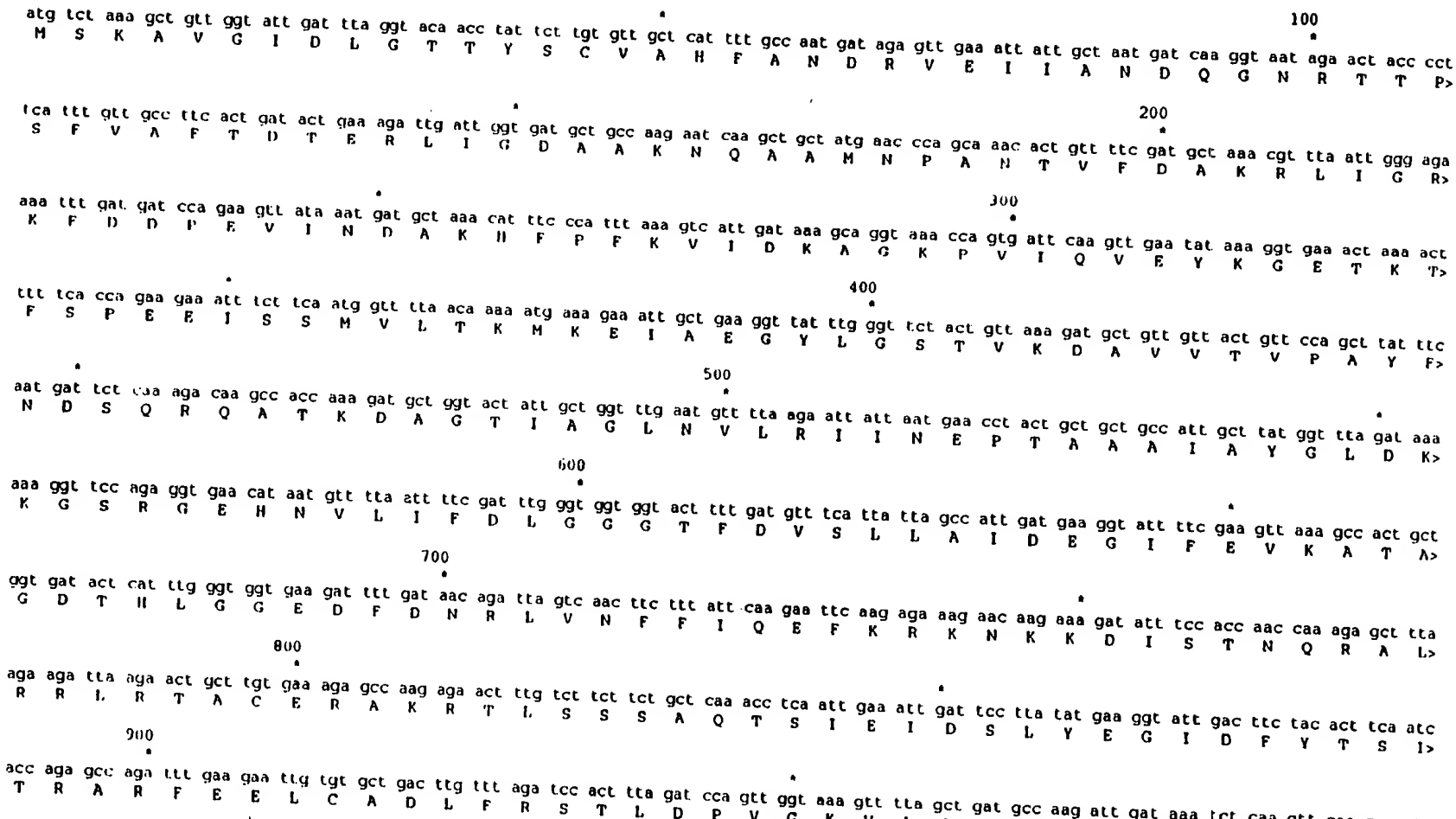
3. A nucleic acid according to claim 2 comprising the nucleotide sequence of SEQ ID No.1 or parts thereof.

4. Composition comprising a nucleic acid according to any of claims 1 to 3.

5. Use of the nucleic acid according to any of claims 1 to 3 for oligonucleotide probes to be used in diagnosis and typing of Candida and Candida related pathologies.

6. Oligonucleotide having a sequence comprised in SEQ ID No. 1 to be used for PCR (polymerase chain reaction) to detect the presence in biological and/or environment samples either of C. albicans or of other Candida species or of yeast-like related microorganisms comprising said gene and/or in a labeled form (radionuclides, biotin, enzymes, etc.) to detect the presence in biological and/or environment samples either of C. albicans or of other related and/or for the C. albicans or related species typing and/or diagnosis and/or as potential antibiotic and/or chemiotherapic targets, or antisense RNA active for Candida species and/or yeast-like related microorganisms coding an homologous sequence.

7. Polypeptide having the aminoacid sequence comprised in the SEQ ID No.1, or having at least a 50% homology with SEQ ID No. 1 or fragments, and/or functional and immunologic homologous thereof.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M S K A V G I D L G T T Y S C V A H F A N D R V E I I A N D Q G N R T T P>
100
tca ttt gtt gcc ttc act gat act gaa aga ttg att ggt gat gct gcc aag aat caa gct gct atg aac cca gca aac act gtt ttc gat gct aaa cgt tta att ggg aga
S F V A F T D T E R L I G D A A K N Q A A M N P A N T V F D A K R L I G R>
200
aaa ttt gat gat cca gaa gtt ata aat gat gct aaa cat ttc cca ttt aaa gtc att gat aaa gca ggt aaa cca gtg att caa gtt gaa tat aaa ggt gaa act aaa act
K F D D P E V I N D A K H F P F K V I D K A G K P V I Q V E Y K G E T K T>
300
ttt tca cca gaa gaa att tct tca atg gtt tta aca aaa atg aaa gaa att gct gaa ggt tat ttg ggt tct act gtt aaa gat gct gtt gtt act gtt cca gct tat ttc
F S P E E I S S M V L T K M K E I A E G Y L G S T V K D A V V T V P A Y F>
400
aat gat tct caa aga caa gcc acc aaa gat gct ggt act att gct ggt ttg aat gtt tta aga att att aat gaa cct act gct gct gcc att gct tat ggt tta gat aaa
N D S Q R Q A T K D A G T I A G L N V L R I I N E P T A A A I A Y G L D K>
500
aaa ggt tcc aga ggt gaa cat aat gtt tta att ttc gat ttg ggt ggt ggt act ttt gat gtt tca tta tta gcc att gat gaa ggt att ttc gaa gtt aaa gcc act gct
K G S R G E H N V L I F D L G G G T F D V S L L A I D E G I F E V K A T A>
600
ggt gat act cat ttg ggt ggt gaa gat ttt gat aac aga tta gtc aac ttc ttt att caa gaa ttc aag aga aag aac aag aaa gat att tcc acc aac caa aga gct tta
G D T H L G G E D F D N R L V N F F I Q E F K R K N K K D I S T N Q R A L>
700
aga aga tta aga act gct tgt gaa aga gcc aag aga act ttg tct tct tct gct caa acc tca att gaa att gat tcc tta tat gaa ggt att gac ttc tac act tca atc
R R L R T A C E R A K R T L S S S A Q T S I E I D S L Y E G I D F Y T S I>
800
acc aga gcc aga ttt gaa gaa ttg tgt gct gac ttg ttt aga tcc act tta gat cca gtt ggt aaa gtt tta gct gat gcc aag att gat aaa tct caa gtt gaa gaa att
T R A R F E E L C A D L F R S T L D P V G K V L A D A K I D K S Q V E E I>
900

FIG. 1

```
1000
gtc ttg gtt ggt ggg tcc acc aga att cca aag att caa aaa ttg gtt tct gat ttc ttt aat ggt aaa gaa ttg aat aaa tct atc aac cct gat gaa gct gtt gct ta
V L V G G S T R I P K I Q K L V S D F F N G K E L N K S I N P D E A V A Y
                                                                    1100
ggt gct gct gtt caa gct gcc att tta act ggt gat act tct tcc aag act caa gat att ttg tta ttg gat gtt gct cca ttg tca tta ggt att gaa act gct ggt gg
G A A V Q A A I L T G D T S S K T Q D I L L L D V A P L S L G I E T A G G
                                                                    1200
atc atg acc aaa ttg att cca aga aat tct act att cca act aag aaa tca gaa act ttc tcc act tat gcc gat aac caa cca ggt gtt ttg att caa gtg ttt gaa gg
I M T K L I P R N S T I P T K K S E T F S T Y A D N Q P G V L I Q V F E
                                                                    1400
gaa aga gct aaa act aaa gat aac aac ttg ttg ggt aaa ttt gaa tta tct ggt att cca cca gct cca aga ggc gtc cct caa att gaa gtt act ttc gat att gat gc
E R A K T K D N N L L G K F E L S G I P P A P R G V P Q I E V T F D I D A
                                                                    1500
aat ggt atc ttg aat gtt tct gct tta gaa aaa ggt act ggt aaa act caa aag att act atc acc aac gat aaa ggt aga tta tcc aaa gaa gaa att gat aaa atg gt
N G I L N V S A L E K G T G K T Q K I T I T N D K G R L S K E E I D K M V
                                                                    1600
agt gaa gct gaa aaa ttc aaa gaa gat gaa aag gaa gct gct aga gtc caa gcc aag aat caa ttg gaa tct tat gct tat tca ttg aaa aac aca atc aat gat ggt
S E A E K F K E E D E K E A A R V Q A K N Q L E S Y A Y S L K N T I N D G
                                                                    1700
gaa atg aaa gat aag att ggt gca gat gat aaa gaa aaa tta act aaa gcc att gat gaa act att tct tgg tta gat gca tct caa gct gct tct act gaa gaa tac gat
E M K D K I G A D D K E K L T K A I D E T I S W L D A S Q A A S T E E Y D
                                                                    1800
gat aaa cgt aaa gaa tta gaa tca gtt gct aat cca atc att agt ggt gct tat ggt gct gcc ggt ggc gct cca ggt ggt gca ggc gga ttc cca ggt gct ggt ggc ttc
D K R K E L E S V A N P I I S G A Y G A A G G A P G G A G G F P G A G G F
                                                                    1900
cca ggt ggt gcc cca ggt ggt ggt cca ggt ggt gct act ggt ggt gaa tct agt gga cca act gtt gaa gaa gtt gat taa
P G G A P G A G G P G G A T G G E S S G P T V E E V D *>
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FIG. 1(cont)

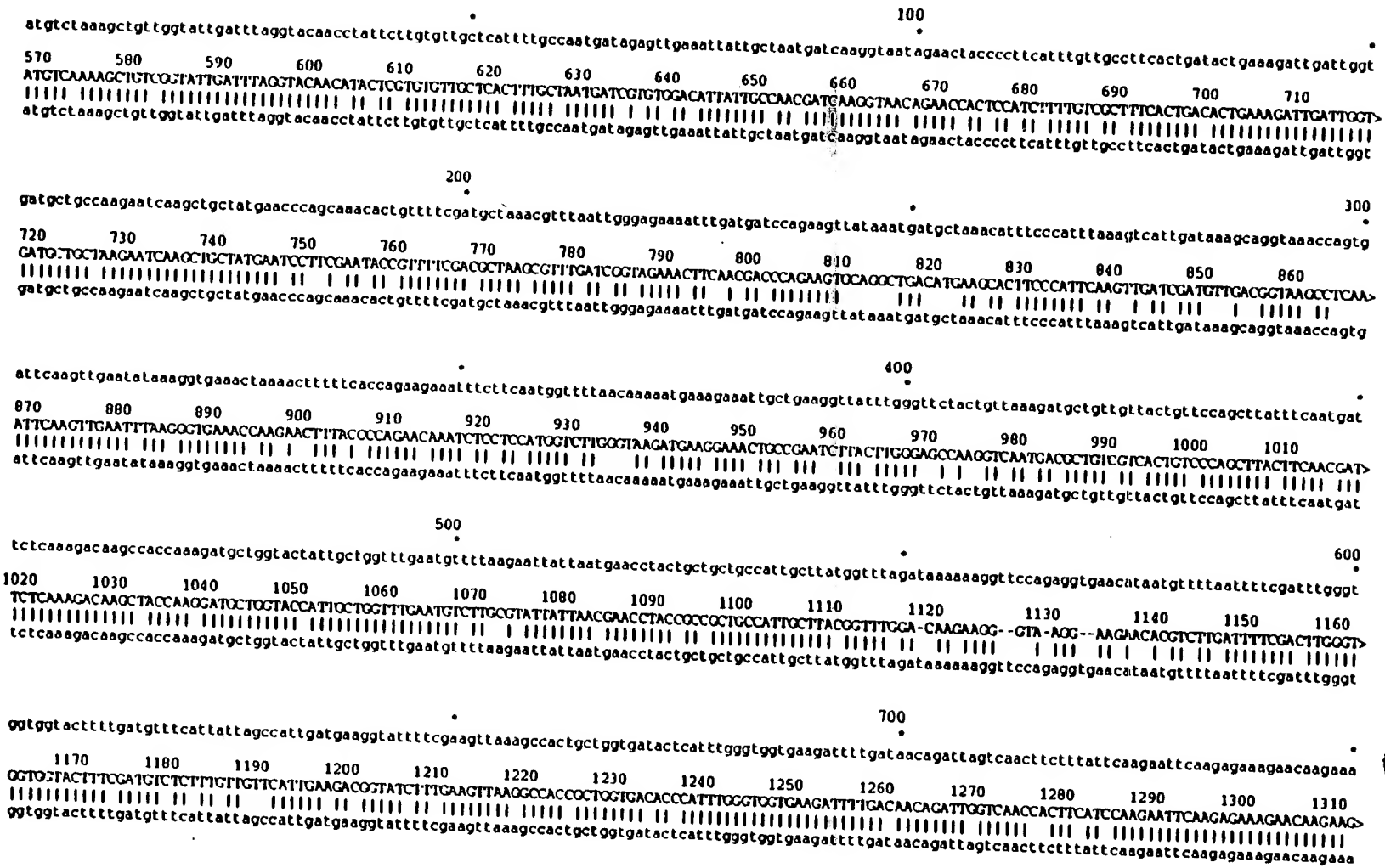


FIG. 2

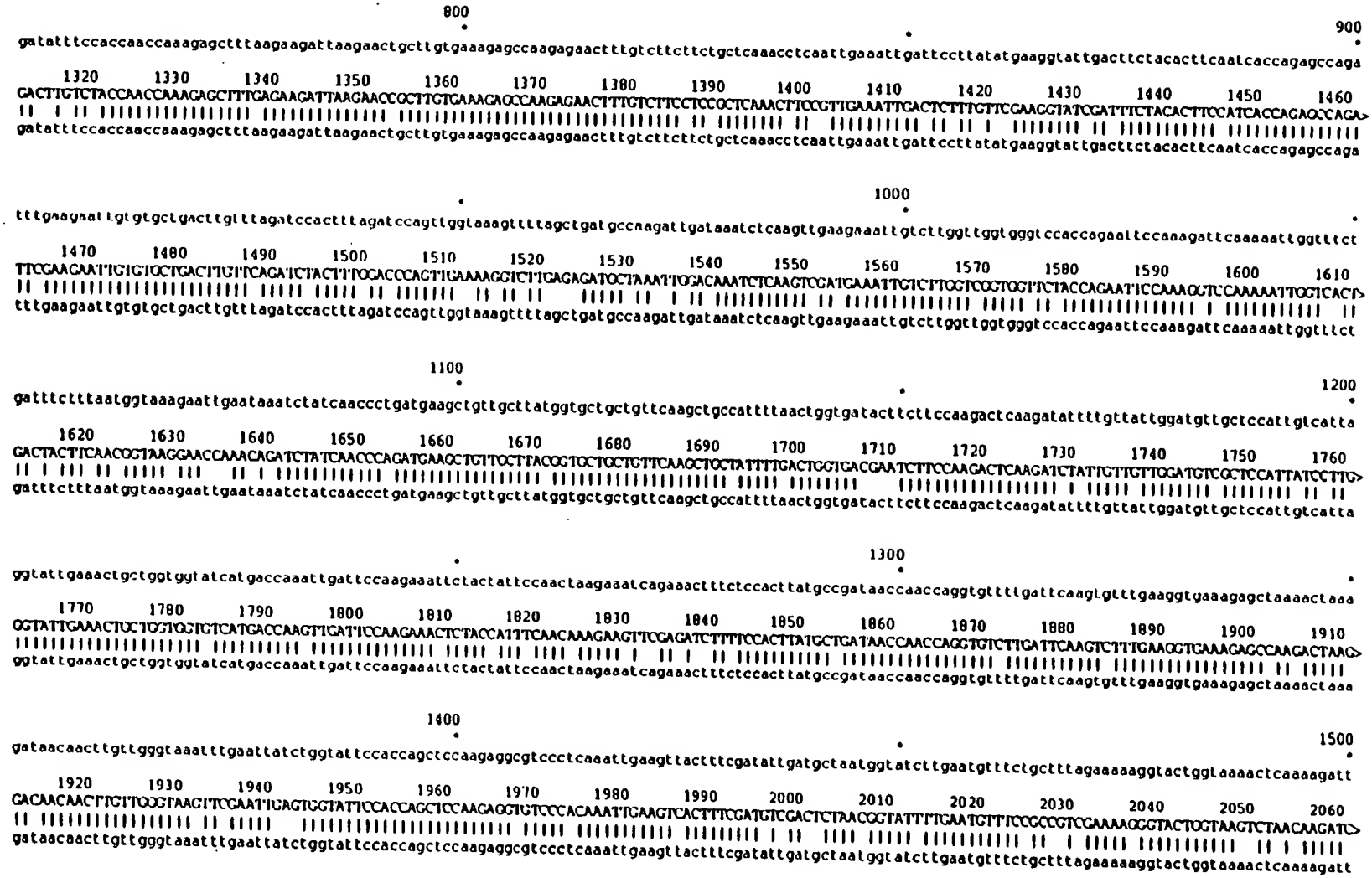


FIG. 2 (cont)

FIG. 2 (cont)

FIG. 3

◁ 3,5 Mbp

◁ 0,7 Mbp

FIG. 4

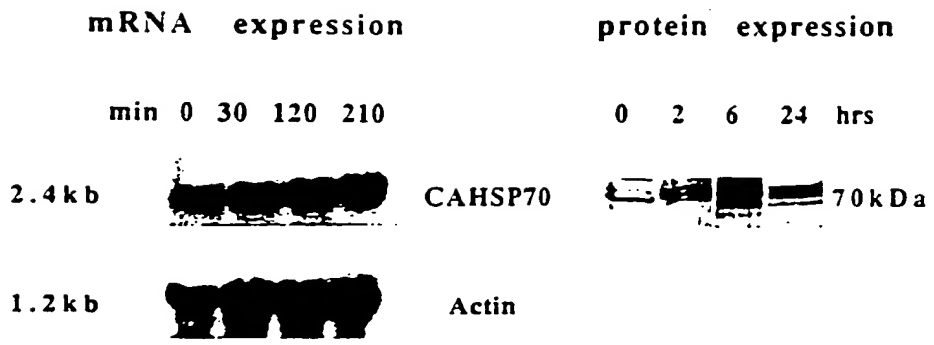
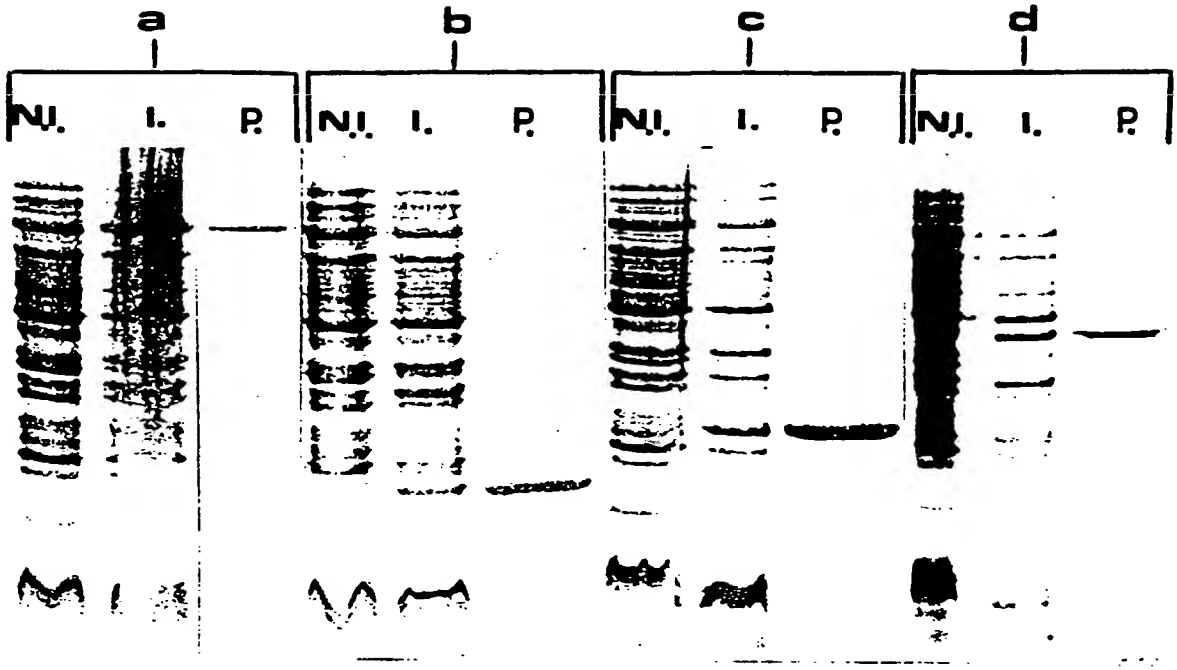
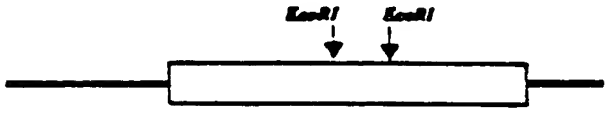

mRNA expression
protein expression
min 0 30 120 210
0 2 6 24 hrs
2.4kb
CAHSP70
70kDa
1.2kb
Actin

FIG. 5

A


a
b
c
d
N.I.
I.
P.
N.I.
I.
P.
N.I.
I.
P.
N.I.
I.
P.

B


EcoRI
EcoRI

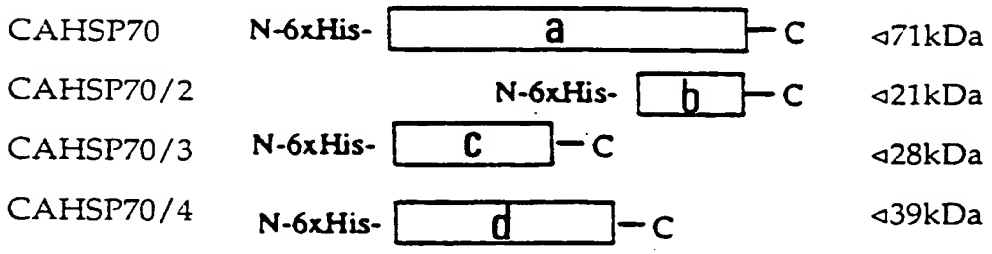

CAHSP70
N-6xHis-
a
C
◁71kDa
CAHSP70/2
N-6xHis-
b
C
◁21kDa
CAHSP70/3
N-6xHis-
c
C
◁28kDa
CAHSP70/4
N-6xHis-
d
C
◁39kDa

FIG. 6

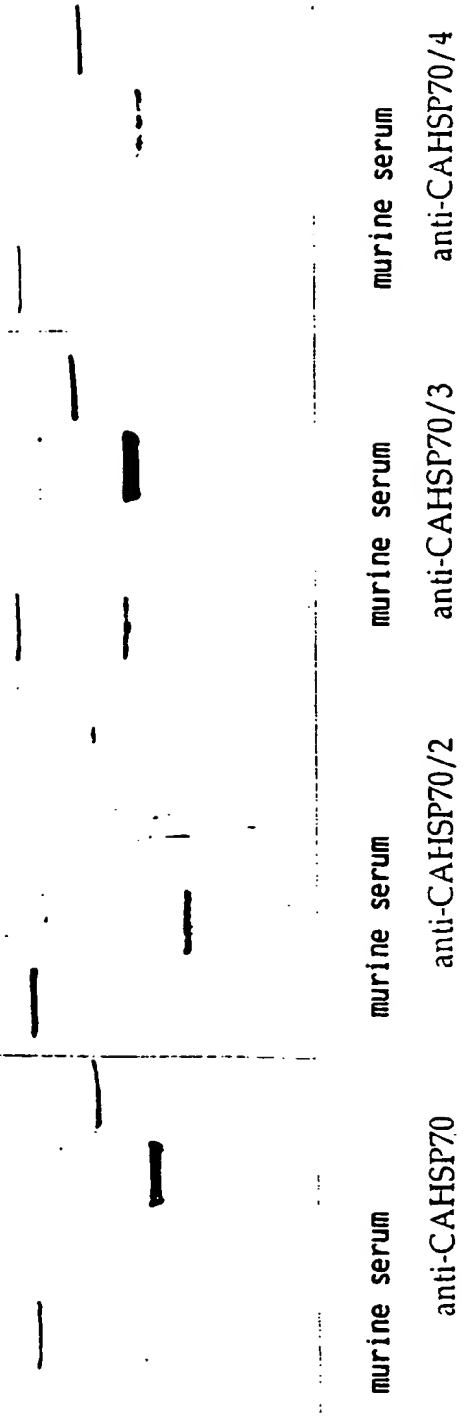

kDa
71
39
28
21
a b c d
murine serum
anti-CAHSP70
anti-CAHSP70/2
anti-CAHSP70/3
anti-CAHSP70/4

FIG. 7

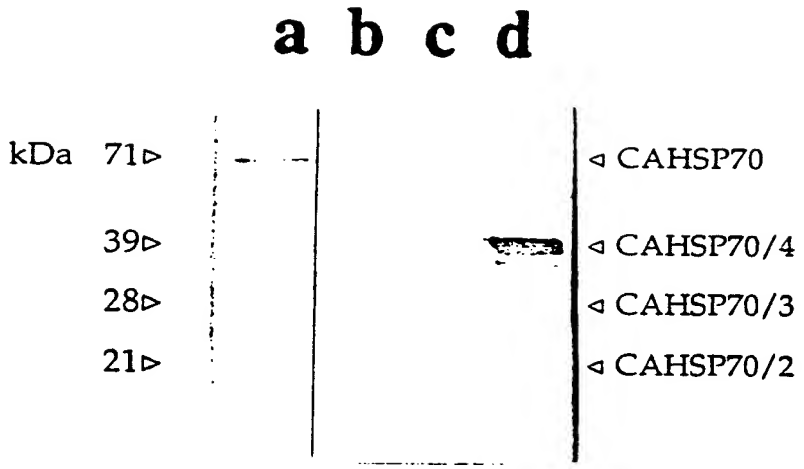
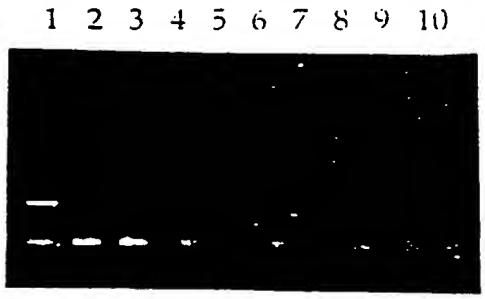


FIG. 8

A



B

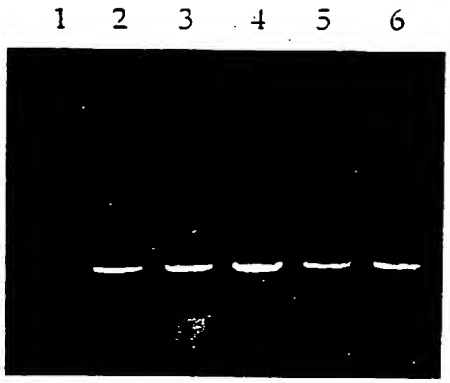


FIG. 9

INTERNATIONAL SEARCH REPORT

Interr[illegible]nal Application No
P[illegible]T 96/00097

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 C12Q1/68 C07K14/40 C07K16/14 G01N33/569
A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUCLEIC ACIDS RESEARCH; vol. 17, no. 2, 25 January 1989, OXFORD GB, pages 805-806, XP002011023 M.R.SLATER AND E.A.CRAIG: "The SSA1 and SSA2 genes of the yeast Saccharomyces cerevisiae" see figure 1 ---	1-4,7,8
A	EP,A,0 406 029 (J.P.BURNIE AND R.C.MATTHEWS) 2 January 1991 see the whole document ---	9-12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 August 1996	28.08.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
/IT 96/00097

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	INFECTION AND IMMUNITY, vol. 63 , no. 10, October 1995, WASHINGTON US, pages 4039-4045, XP002011024 R.LA VALLE ET AL.: "Molecular cloning and expression of a 70-kilodalton heat shock protein of Candida albicans" see the whole document	1-12
A	JOURNAL OF MOLECULAR EVOLUTION, vol. 38, no. 1, January 1994, pages 1-17, XP000578395 W.R.BOORSTEIN ET AL.: "Molecular evolution of the HSP70 multigene family" see table 3	1-4,7,8

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 12, insofar as it relates to in vivo uses, is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IT 96/00097

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Futher Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted] on patent family members

Inter[redacted]nal Application No
[redacted]IT 96/00097

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